Metrics for Optimization of Cell Selection Technologies
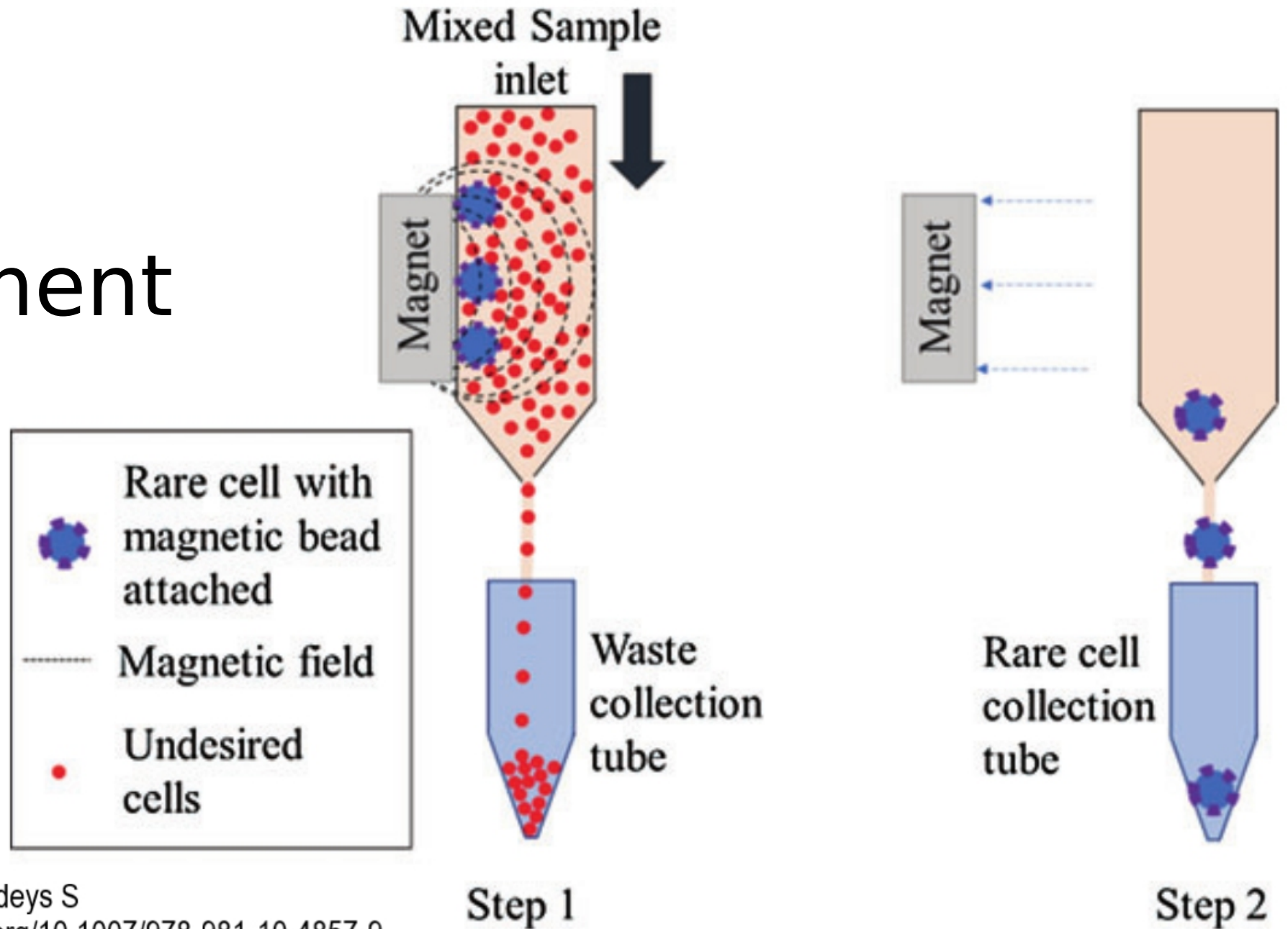
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Diether Recktenwald, Desatoya LLC, Reno NV

http://www.desatoya.com

Diether@desatoya.com, +1-408-658-6074

Rare Cell Enrichment

Legend:
- Rare cell with magnetic bead attached
- ---------- Magnetic field
- • Undesired cells

Mixed Sample inlet

Magnet

Waste collection tube

Step 1

Magnet

Rare cell collection tube

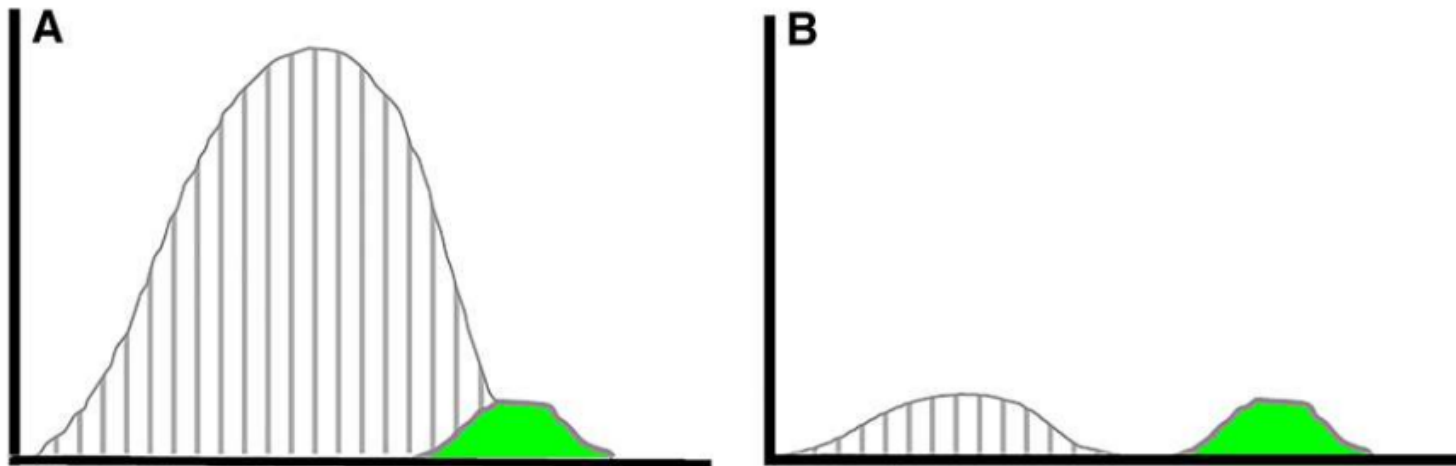**Step 2**

Benefit of pre-enrichment before FACS™

Figure 11. Improvement of population discrimination after pre-enrichment. Cytometer histograms of unwanted (gray lines) and wanted (solid green) populations. (A) A large excess of an unwanted population may create substantial overlap with the target population, making it impossible to achieve a good single-cell sort. (B) After a pre-enrichment bulk sort, which removes most of the unwanted population a good discrimination between the two populations can be achieved.

Figure provided by Dr. Charlotte Esser, Universität Düsseldorf, Germany

Metrics to Optimize the MACS system

$$\text{Enrichment rate } (f_E) = \frac{\% \text{ neg. cells in orig. sample}}{\% \text{ pos. cells in orig. sample}} \times \frac{\% \text{ pos. cells in pos. fraction}}{\% \text{ neg. cells in pos. fraction}}$$

$$\text{Depletion rate } (f_D) = \frac{\% \text{ pos. cells in orig. sample}}{\% \text{ neg. cells in orig. sample}} \times \frac{\% \text{ neg. cells in neg. fraction}}{\% \text{ pos. cells in neg. fraction}}$$

"Miltenyi S, Schmitz J. High Gradient Magnetic Cell Sorting, pages 218ff. in Radbruch A (Ed.) Flow Cytometry and Cell Sorting. 2nd edition. Springer Lab Manual 1999"

Miltenyi S, Schmitz J (1999)

Cell Separation Arithmetic

$$P_+ = \frac{f_+ \cdot Y_+}{f_+ \cdot Y_+ + f_- \cdot Y_-}$$

$$f_- = 1.0 - f_+$$

$$P_+ = \frac{f_+ \cdot Y_+}{f_+ \cdot Y_+ + (1.0 - f_+) \cdot Y_-}$$

The purity of the final enriched suspension is the number of positive cells divided by the total number of cells (positives+negatives) as expressed in the equations above.

I like to describe the efficiency of a cell separation process in terms of Y_+ and Y_-. Y_- can be further divided into the contributions of different cell types, and the information can be obtained from a detailed subset analysis of the fractions. For an ideal positive enrichment Y_- is 0.0 and Y_+ is 1.0. Depletion can be described in an analogous way.

Example of a positive CD34 enrichment with $Y+= 0.9$ $Y-=0.001$ and $f+=0.002$:
$P+= 0.002*0.9/(0.002*0.9+(1-0.002)*0.001) = 0.643$ Purity= 64.3%

f_- = fraction of negatives (%/100) in original pre-enrichment suspension
f_+ = fraction of positives in original pre-enrichment suspension
P_+ = fraction of positives in final enriched suspension
Y_+ = probability of positives being retained in enrichment process
Y_- = probability of negatives being retained in enrichment process

Link to Calculation Spreadsheet

Table 2 Therapeutic Scale Isolation of CD34 Progenitor Cells from Leukapheresis Harvest of Filgrastim-Stimulated Patients

	CD34 cells[a]			Log Depletion		
	Original	Purity	Yield	CD3 T cells	CD14 monocytes	CD20 B cells
Average	0.8%	95%	92%	4.5	3.9	4.1
SD	0.3	4	5	0.2	0.2	0.3
Range	0.4–1.3%	88–99%	84–99%	4.1–4.7	3.4–4.4	3.6–4.7

[a] $n = 10$ for CD34 cells data and 5 for log depletion data.

Kantor, A. B., Gibbons, I., Miltenyi, S., & Schmitz, J. (1998).
Magnetic cell sorting with colloidal superparamagnetic particles.
In Recktenwald D, Radbruch A edts,Cell separation methods and applications, 153-173.

Table 1. Example of sort performance metrics. The values for purity, yield, and the fraction of positive cells in the original sample are measured and the negative logarithm of negYieldFraction, –log(Y–) (the underlying equations are detailed in the text (Section II.1.1) and the logarithm of Fe [84] are calculated

Purity (%)	Yield (%)	Orig (%)	–log(Y–)	log(Fe)
95	100	50	1.28	1.28
95	90	50	1.32	1.28
95	10	50	2.28	1.28
99	90	1	4.04	3.99
95.6	90	0.1	4.38	4.34

Calculation of performance of cell selection systems from published data.

System	P%	Y+%	f+%	-log(Y-)	log(Fe)
Droplet Sorters					
MoFloXDP CD19 (BC WP)	99.91	90	14.84	3.85	3.80
Aria CD3 (BC WP)	94.61	80	0.1	4.34	4.24
Microfluidic Sorters					
NanoCellect Beads	55.6	80	0.63	2.39	2.30
NavazAA2015 HeLa cells	92.6	84	51.2	1.15	1.08
Hwy1 CD34	94.6	61	5.19	2.72	2.51
Hwy1 CD19	96.6	70	8.35	2.65	2.49
Hwy1 CD3	99.5	70	63.5	2.21	2.06
Hwy1 CD14	96.5	70	20.2	2.19	2.04
Hwy1 CD3 wh. Blood	55.7	70	0.3	2.78	2.62
Bulk Magnetic Separation					
CliniMACS CRTC paper	85	79	0.09	3.9	3.80
EasySep CD4	96	55	28	2.1	1.79
Imag CD4	89.3	55	20.3	1.8	1.52
QuadT KG1a CD34	97.5	92	20	2.2	2.19
SCT T-cells	96.9	90	52.5	1.5	1.45
SCT Easysep CD34	95	75	0.08	4.5	4.38